Influence of seed nitrogen content on seedling growth and recovery from nitrogen stress

E.R. Naegle[1], J.W. Burton[1,2], T.E. Carter[1,2] & T.W. Rufty[1,3]

[1]*Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620, USA.* [2]*U.S. Department of Agriculture, Agricultural Research Service, 3127 Ligon St. Raleigh, NC 27607.* [3]*Corresponding author**

Abstract

In low fertility conditions, germinating seedlings are heavily dependent upon N from seed reserves for growth and development. Experiments were conducted to examine the influence of seed N content and mobilization on leaf development and the ability to take up nitrate and up-regulate leaf growth processes during recovery from the period of N deprivation. Genetically homozygous soybean lines with a range of seed N contents were grown in hydroponics culture without an external N source. Microscopic analyses of the apical meristem and plant exposure to labeled ^{15}N-nitrate allowed precise descriptions of changes in leaf initiation and nitrate uptake during recovery. In seedlings grown without N, inhibitions of leaf expansion and leaf initiation were detectible in the 2nd week after germination, and both were inversely related to the amount of N released from the seed. The S/R ratio decreased, indicating early N stress, but the S/R adjustments were not proportional to the degree of N stress as occurs with older plants, suggesting limitations in C availability. When external N was supplied to plants after 15 or 23 days of N deprivation, the induction period for nitrate uptake was longer than that normally observed with N-replete seedlings, and rates of growth during recovery from the N stress were correlated with initial seed N contents. The inhibition of individual leaf expansion was released only if leaves were at an early developmental stage. Leaf initiation at the apical meristem immediately responded to the presence of external nitrate, with initiation rates approaching those for $+$N controls. The results indicate that seedling vigor in infertile conditions and the ability to respond to available N are strongly influenced by seed resources.

Introduction

Young plants often encounter N stress when growing in highly weathered soils like those found in the southeastern United States, tropical America, and southeast Asia. The soils typically are classified as oxisols and ultisols and have low levels of inorganic N and organic matter (Buol et al., 1973), and low N availability for plant uptake. The ability of seedlings to become established and survive in low fertility conditions depends, in large part, upon phenotypic plasticity (Bradshaw, 1965; Sultan, 2003), i.e. how well they adjust to or tolerate nutritional stress and take advantage of N acquisition opportunities (Caldwell, 1994; Grime et al., 1986).

A major factor controlling seedling growth responses in low fertility conditions may be the N content of seed. Seed reserves may be the primary and, in some cases, the only source of N that can drive growth processes. A key regulatory role for seed N was implied by studies showing that higher seed nutrition was associated with

increased seedling vigor when fertility was low (Bulsani and Warner, 1980; Hara and Toriyama, 1998; Nedel et al., 1996; Parrish and Bazzaz 1985; Ries, 1971; Tungate et al., 2002; Welch, 1999). There has not been, however, a detailed study of the relationship between the seed N resource level and physiological responses of seedlings.

A fundamental response in coping with N deficiencies is to adjust leaf development. From many studies with older plants in agronomic systems, it is known that N deficiencies result in plants with fewer and smaller leaves (e.g., Greenwood, 1976; Watson, 1947). Decreasing or increasing the external N supply in hydroponics experiments initiates coordinated changes in leaf production and canopy leaf area expansion (Tolley-Henry and Raper, 1986), which implies dependency of leaf development on N availability. Also, inhibition of expansion of individual leaves has been observed to be the first growth response for N-stressed plants (Radin and Boyer 1982; Rufty 1998).

Within a functional framework, adjustments in leaf development are paradoxical. On one hand, the ability of seedlings to sustain leaf area expansion under N stress increases photosynthetic capacity and enhances spatial competitiveness. On the other, inhibition of leaf development is a key physiological step leading to increased allocation of dry matter to the root and increased root growth (Brouwer, 1962; Ingestad and Lund, 1979; Rufty 1998). Lowering of shoot to root ratios is thought to be a fundamental strategy among plant species, promoting root exploration of soil and subsequent acquisition of nutrients (Chapin, 1991).

This study was conducted to examine the influence of different seed N contents on growth of seedlings deprived of and then supplied with external N. Experiments focused on the relationship between seed N mobilization and degree of restriction of leaf initiation and expansion, and the extent that seed N modified the ability of seedlings to take up nitrate and up-regulate leaf growth processes. The experiments were conducted under highly controlled conditions using genotypes of soybean. As a result of plant breeding for specific markets, genetically stable soybean lines have been selected with differing seed N contents (Burton, 1984). The lines were derived from a narrow genetic base (Gizlice et al., 1994) and have similar growth rates when ample external N is available. This allowed evaluation of the relationship between seed N and seedling response with minimal confounding effects.

Materials and methods

Sixteen soybean (*Glycine max* [L.] Merr.) lines (Clifford, Dillon, Haskell, Holladay, Prolina, Ransom, Young, NC-101, NC-104, NC-105, NC-106, NC-110, NC-111, NC-112, N93-1264, and D68-0099) were selected from plant breeding databases as likely to have a range of seed N contents, and examined in initial experiments. Seeds were wrapped in germination paper moistened with 0.1 mM $CaSO_4$ and placed in a dark germination chamber at 28 °C and 98% relative humidity for 3 days. Seedlings with roots 8–12 cm in length were selected and placed into 50-L continuous-flow hydroponics systems. The systems were located in a walk-in growth chamber in the Southeastern Plant Environmental Laboratory at North Carolina State University. The chamber was programmed for a day/night temperature of 26/22 °C and a 9-h light period with cool white fluorescent and incandescent light (PPFD of 550 ± 50 μmol m^{-2}s^{-1}). A 3-h night interruption with incandescent light (PPFD of 30.5 ± 3.4 μmol m^{-2}s^{-1} and photomorphogenic irradiance of 11 ± 1 Wm^{-2}) was used to repress flowering.

The nutrient solution temperature was maintained at 24 ± 0.5 °C, and pH at 6.0 ± 0.1 with automated monitoring and additions of KOH and H_2SO_4. The complete nutrient solution composition was: 200 μM KH_2PO_4, 600 μM KNO_3, 300 μM $MgSO_4$, 800 μM $CaSO_4$, 19 μM H_3BO_3, 3.7 μM $MnCl_2 \cdot H_2O$, 317 nM $ZnSO_4$, 132 nM $CuSO_4$, 50 nM H_2MoO_4, and 35.8 μM Fe as Fe-Sequestrene. When plants were grown without an external N source, KNO_3 was replaced with 300 μM K_2SO_4. Nutrients were monitored and adjusted so that depletion was minimized to less than 30% of the initial solution concentrations.

Seedlings from the 16 lines were grown for 27 days in the presence or absence of N. At the end of the experiment, eight plants of each line were harvested, dried at 60 °C, and weighed.

Three of the 16 lines, NC-106, Young, and D68-0099, representing a wide variation in seed N content and growth response, were selected for more detailed experiments.

Three types of experiments were conducted with the three soybean lines. In one, plants were grown in the complete nutrient solution with or without N for 27 days, as before. Four randomly selected plants from each treatment were harvested every 2–3 days. In a second type of experiment, groups of plants were grown in −N solutions for either 14 or 23 days, at which point KNO_3 was added to the nutrient solution to establish a N concentration of 600 μM. Four randomly selected plants of each line then were harvested at 4 or 5-day intervals over a 15-day recovery period. In these experiments, shoot, root, and cotyledon tissues were separated at harvest, and leaves were counted. Shoot apical meristems were examined using a dissecting microscope to detect primordia emerging from the apical dome; thus, leaf initiation estimates include macroscopic and microscopic leaves. Areas of individual leaves ≥ 2.00 cm^2 were measured with a Li-Cor 3100 leaf area meter (Li-Cor Instruments, Lincoln, NE). Tissues were oven dried at 60 °C, weighed, and ground. Tissue N was measured using a CHN Elemental Analyzer (Model 2400, Perkin–Elmer Corp., Norwalk, CT, USA).

A third type of experiment was conducted using ^{15}N to characterize the capacity for nitrate uptake among the three soybean lines. Seedlings of NC-106, Young, and D68-0099 were grown in −N nutrient solutions as described previously for 15 days, at which time KNO_3 was added to the solution 3 h into the light period to establish a N concentration of 600 μM. At the time of initial exposure to nitrate and after 4, 12, 24, 48, and 77 h four plants of each line were removed from the hydroponics units and placed into 4-L beakers containing similar aerated nutrient solutions, but with 98 atm. % ^{15}N labeled nitrate for 3 h uptake periods. Additional sets of plants were exposed to solutions containing 10 atm. % ^{15}N- nitrate for 5 h during the light period on days 4, 5, 7, 9, 11, and 13 after the initial nitrate addition. The ^{15}N treatments were within the same growth chamber as the hydroponics units. Immediately before and after exposure to ^{15}N, plant roots were dipped 5 times in 1.0 mM

$CaSO_4$ to remove apoplastic nitrate. After ^{15}N exposures, roots and shoots were separated, dried, weighed, and ground. Ground tissues were analyzed for total N and ^{15}N enrichment using elemental N analysis and ratio mass spectroscopy.

Statistical analysis was performed using ANOVA. Significant differences from treatments were determined at $P \le 0.05$, using Tukey's HSD procedure. Curves in figures were determined using the regression curve-fitting function of Sigmaplot (http://www.spss.com, SPSS Inc., Chicago, IL 60611). From an appropriate category (e.g. polynomial, exponential decay, hyperbola, exponential rise to max, or sigmoidal), the equation with the highest r^2 was used. The results of a single series of experiments were presented in graphs, but the experiments were repeated and responses always were similar.

Results

Initial experiments were conducted to resolve the general relationship between seed N content and growth. Sixteen soybean lines were grown for an extended period without external N. After 27 days, plant dry weights ranged from 300 to 1000 mg and the weights were positively correlated with original seed N contents (Figure 1). This contrasted with the much lower correlation between seedling growth and seed dry weight ($r^2 = 0.36$, data not shown). Thus, the positive seed N correlation with growth could not be attributed simply to higher total seed reserves. As the experiment progressed, all of the seedlings exhibited typical N deficiency symptoms including leaf chlorosis and senescence of older leaves. The timing and severity of visual Nstress symptoms were inversely related with the initial seed N content.

The Nstress response

To more thoroughly characterize growth responses, three soybean lines were selected from the initial group (NC-106, Young, and D68-0099) that had a wide range of seed N contents, 13.5–6.4 mg N $seed^{-1}$ (Table 1). When N was included in nutrient solutions, plant dry weight accumulation (Figure 2A), leaf area expansion
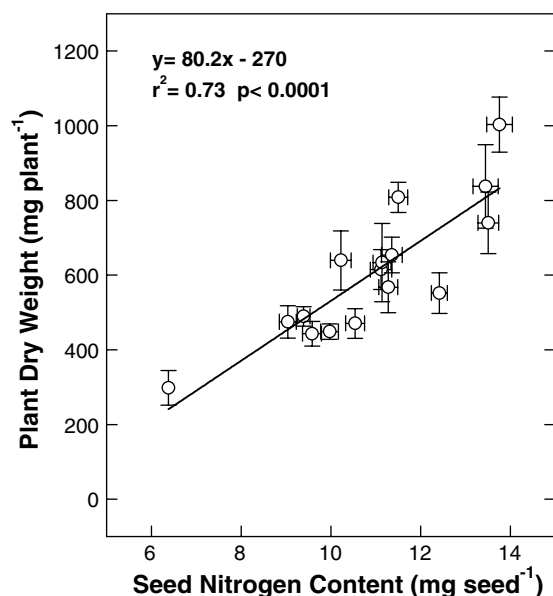
Figure 1. Relationship between seed N content and seedling dry weight of 16 soybean lines after 27 days of growth without an external N supply. Seed N contents are means of 50 seeds, and dry weights are means of 8 plants. Day 0 is the day seeds were first exposed to water; seedlings were placed in hydroponics on day 3. Vertical and horizontal bars represent standard error of the mean.

Table 1. Seed weight, N concentration, and N content (weight × N concentration) of three soybean lines

Line	Dry Weight (mg seed^{-1})	N Concentration (%N)	Seed N Content (mg seed^{-1})
1. NC-106-H (High)	182 a*	7.40 a	13.5
2. Young-M (Medium)	155 b	6.25 b	9.7
3. D68-0099-L (Low)	117 c	5.45 c	6.4

Seed weights are a mean of 50 seeds for each line.
*Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using Tukey's HSD procedure.

(Figure 2B), and N content (data not shown) were not statistically different among the lines after 27 days. Thus, growth potential among the lines was similar, and responses in the absence of external N could be related to N content of the seed with a degree of confidence. For visual clarity, plotted lines for +N control plants were combined in remaining graphs.

Exposure of the seedlings to solutions without external N led to a consistent response pattern, as growth restrictions were greater with lower seed N. Growth differences began to appear after the 2nd week, and there was a three-fold difference in dry weight and a two-fold difference in leaf area between the high and low seed N lines at 27 days (Figure 2).
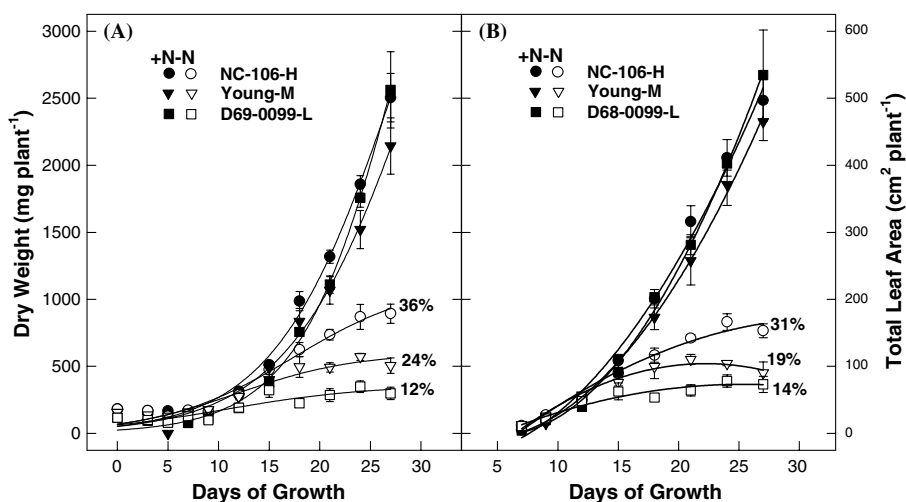

Figure 2. Dry weight (A) and total leaf area (B) of three soybean lines with differing seed N contents after 27 days of growth. Decrease in leaf area of −N Young after day 21 was due to leaf abscission. Insets represent data for −N plants expressed as a % of +N plants at day 27. Vertical bars represent standard error of the mean.
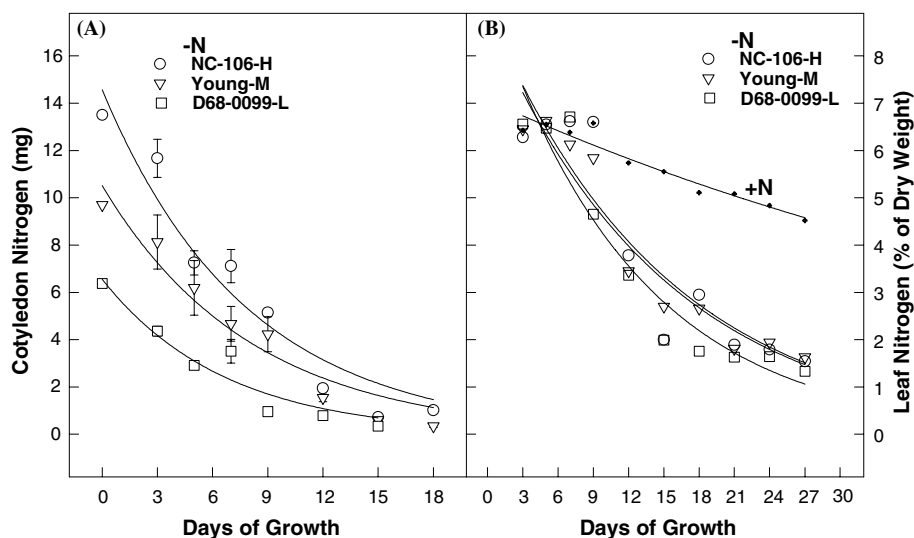
Figure 3. Exponential decrease in cotyledon N content (A) and N concentration of leaves (B) in −N plants +N data for the three soybean lines were averaged and plotted as a single line. Vertical bars represent mean standard error.
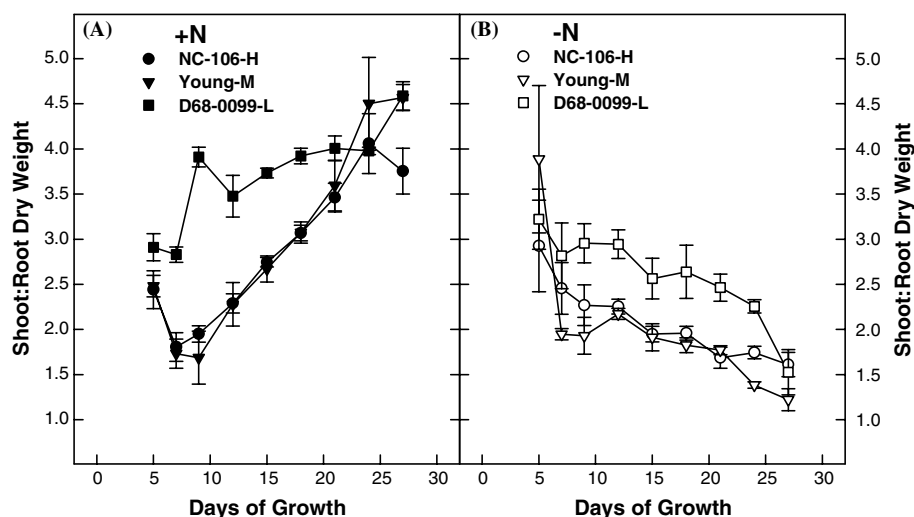

Figure 4. Shoot to root dry weight ratios for plants grown in +N (A) or −N (B) treatments. Vertical bars represent standard error of the mean.

When seedlings were grown without an external N supply, they were entirely dependent on N mobilized from the seed. Nitrogen release from the cotyledons followed a similar exponential decay pattern for the three soybean lines, with nearly all N mobilized by day 15 (Figure 3A). Thus, the amounts of N mobilized to vegetative tissues reflected initial differences in seed N. The dependence on seed N reserves resulted in a steady decline in % N in the seed-

lings compared to controls, as shown for leaf tissues (Figure 3B).

Without external N, shoot growth was limited more than root growth, leading to lowering of S/R ratios. The S/R ratios of +N control plants typically declined initially due to early root growth, but the ratios then increased steadily over time (Figure 4A), the normal pattern expected during the vegetative growth phase (Russell, 1977). In contrast, with −N plants the
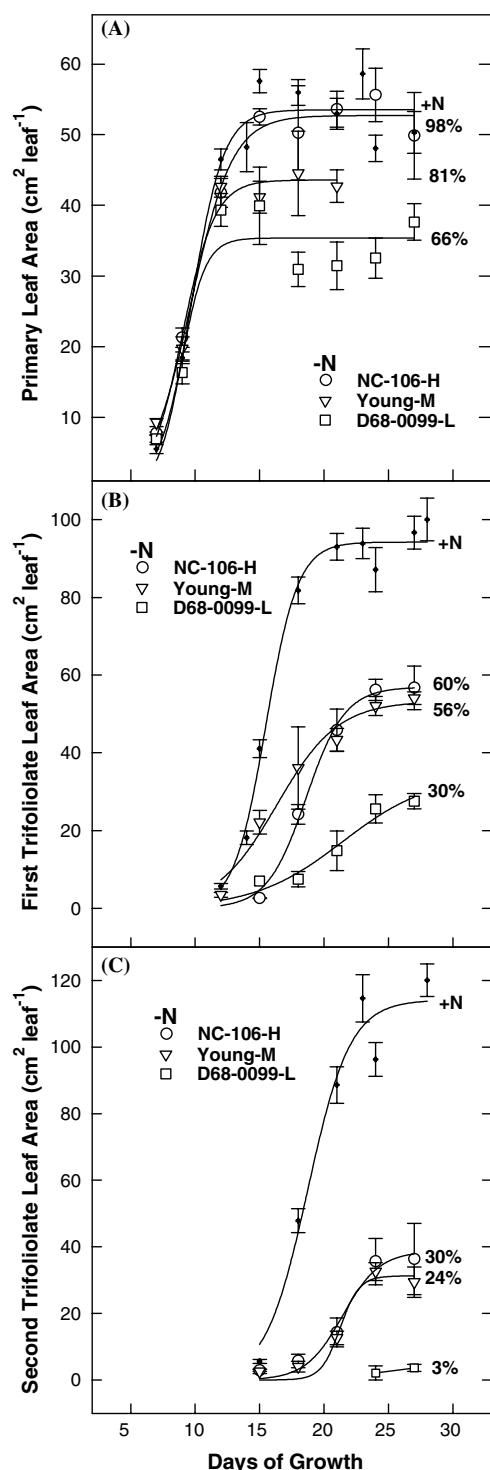
Figure 5. Leaf area of primary (A), first trifoliolate (B), and second trifoliolate (C) leaves over time. +N data for the three soybean lines were averaged and plotted as a single line, and percents were calculated from the +N averages. Vertical bars represent standard error of the mean.
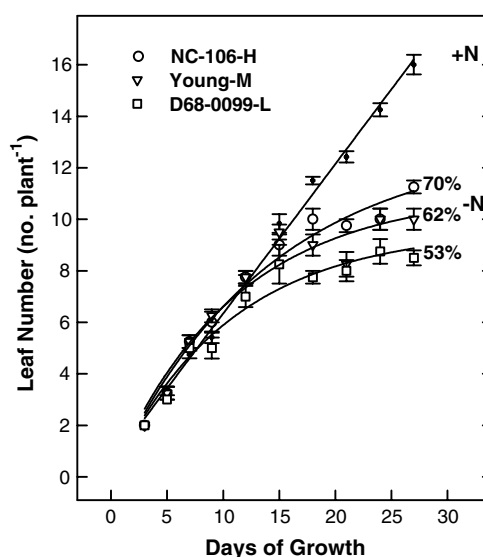

Figure 6. Total leaf number of plants over time, excluding primary leaves. +N data for the three lines were averaged and plotted as a single line. Insets are leaf number of −N plants expressed as a percent of +N average at 27 days. Vertical bars represent standard error of the mean.

Table 2. Number of leaves initiated per day

Line	+N	−N	N Recovery	
			−N for 14 days	−N for 23 days
1. NC-106-H	0.50 a*	0.15 b	0.60 c	0.43 d
2. Young-M	0.51 a	0.07 b	0.47 a	0.52 a
3. D68-0099-L	0.50 a	0.05 b	0.50 a	0.27 c

Slopes of each soybean line were the average of four linear regressions (all $r^2 > 0.95$) from 15 to 27 days (Figure 6) and during recovery periods (Figure 9B).
*Values in the same row followed by the same letter are not significantly different at $P \leq 0.05$ using Tukey's HSD procedure.

restriction of shoot growth led to a steady decline in S/R ratios with time (Figure 4B). An unusual observation was that the decline was not more pronounced with lower seed N availability and increasing degree of N stress. The S/R ratio was highest with D68, the low seed N line, and similar for the other two lines.

Individual leaf expansion was strongly affected by seed N. Expansion of primary leaves of NC-106, for example, was similar to the +N control, but that of Young and D68, the middle and low seed N genotypes, was 81 and 66% of controls at
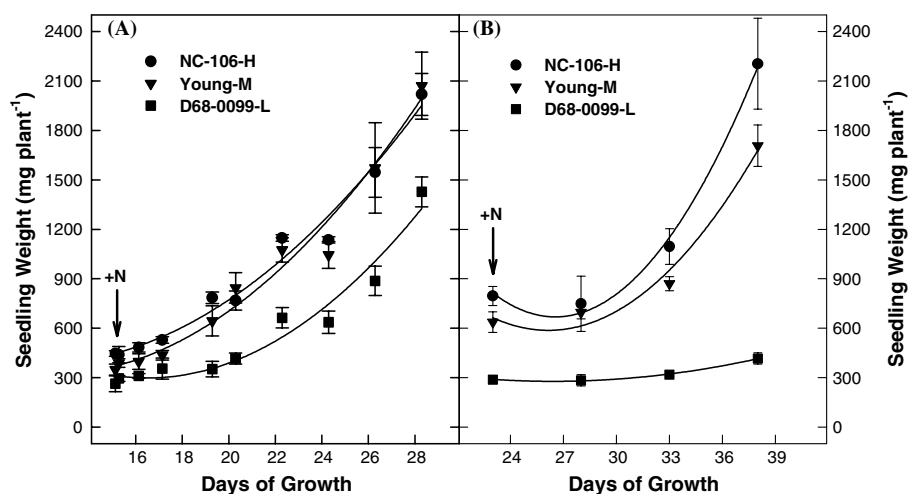
Figure 7. Dry weight accumulation of plants supplied with nitrate after 15 (A) or 23 (B) days of growth without external N. Vertical bars represent standard error of the mean.

full expansion, respectively (Figure 5A). The response pattern repeated in the first and second trifoliolate leaves, as the degree of restriction became greater with lower seed N (Figures 5B and 5C).

Microscopic examination of apical meristems revealed that fewer leaves were being initiated in the −N plants by the end of the 2nd week, and the degree of suppression was influenced by the level of seed N (Figure 6, Table 2). The total number of leaves initiated by day 27 ranged from 11.3 to 8.5 in the high, NC-106, and low, D68, seed N genotypes, respectively.

Recovery from N stress

External nitrate was supplied to N deprived seedlings on days 15 or 23 to determine if seed N had an impact on the plant's ability to recover. The results show that higher seed N was associated with more rapid growth during the recovery phase (Figure 7). Growth of the low seed N line lagged noticeably and was minimal when N was supplied on day 23 (Figure 7B).

Nitrate uptake rates were measured during the recovery period using ^{15}N. With seedlings first supplied nitrate on day 15, uptake rates g^{-1} root increased for about 4–5 days and then stabilized (Figure 8A), presumably reflecting induction of the nitrate uptake system (see discussion below). The low seed N line, D68, initially had a some-

what slower uptake rate compared to the other two lines, but had a slightly higher uptake rate when maximum rates were obtained. The rates of uptake by the seedlings stabilized much higher than those of +N control plants continually exposed to nitrate in solution (2–2.5 mg g^{-1} vs 0.7 mg g^{-1}). Translocation of ^{15}N to the shoot mirrored the increases in uptake as the uptake rate increased (data not shown). After that time, about 70% of the absorbed ^{15}N was found in the shoot for the three soybean lines, so there was no indication of a separate seed N effect on the translocation process. With plants supplied nitrate on day 23, nitrate uptake rates g^{-1} root (estimated from total N accumulation, Figure 8B) steadily increased over the 2-week recovery period, but rates for the low seed N line were lower.

Even though nitrate uptake rates were not maximized for several days, canopy leaf areas increased rapidly when N was supplied to the N-stressed seedlings (Figure 9A). The areas of NC-106 and Young leaves were only about 10–15% lower than the +N controls 2 weeks after external nitrate was supplied (at 27 days, see Figure 2B). Leaf area expansion of D68 was slower than the other genotypes, particularly when nitrate was supplied after 23 days. Leaf initiation responded immediately to the external N supply, as two leaves were initiated during the first 4 days in all three soybean lines (Figure 9B), a rate similar to the controls (Table 2). The same response
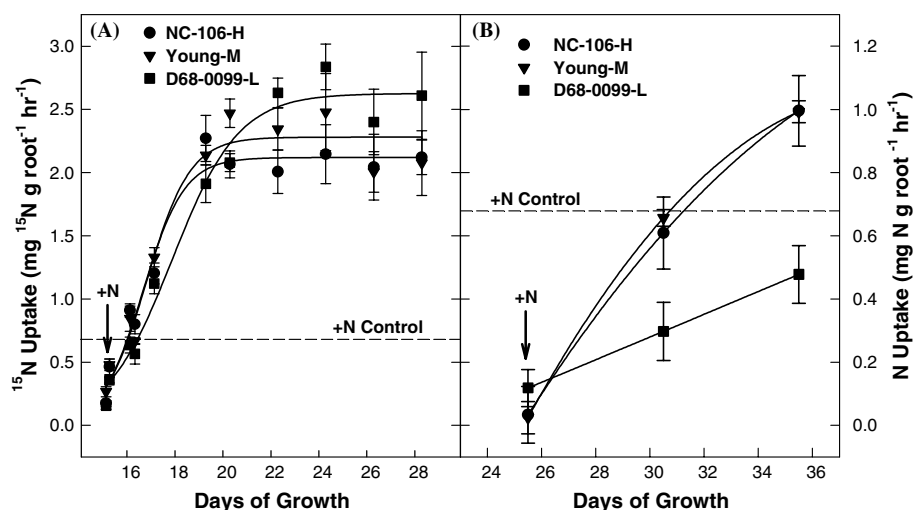
Figure 8. Nitrogen uptake by plants deprived of external N for 15 (A) or 23 (B) days. Note difference in vertical scales. Dashed lines were included to allow comparison with average N uptake rates of the three soybean lines supplied $+N$ throughout. Vertical bars represent standard error of the mean.
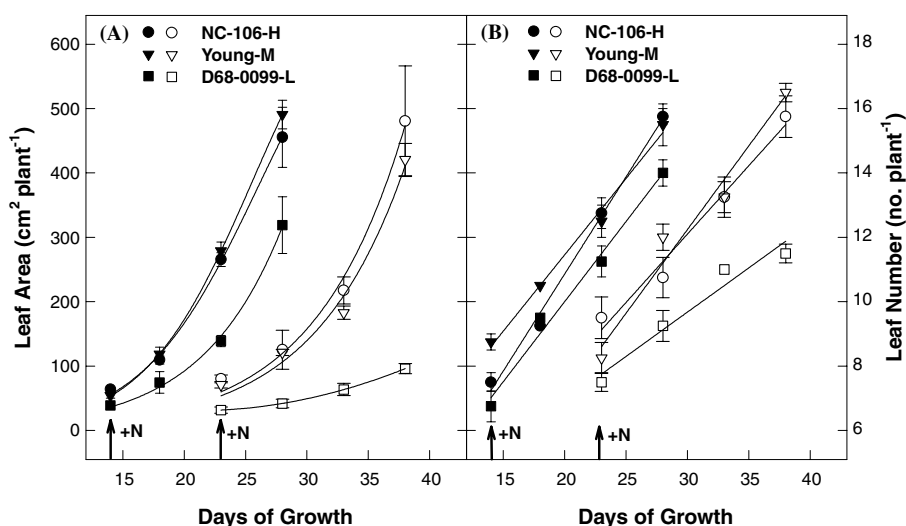

Figure 9. Total leaf area (A) and leaf number (B) of plants grown without N for 14 d (closed) or 23 days (open) and then exposed to $+N$ solutions. Vertical bars represent standard error of the mean.

occurred when nitrate was supplied on day 23 in the two higher seed N lines, with slower initiation occurring with the low seed N line.

Expansion of individual leaves was a function of the stage of leaf development at the time external N was supplied. This was demonstrated with the data for the 1st and 2nd trifoliolate leaves of NC-106, where leaf expansions were plotted against those for plants continually exposed or deprived of N. Supplying external N at day 14,

for example, increased the expansion of leaves at both stem positions compared to the $-N$ treatment, but expansion of the 2nd trifoliolate leaf increased closer to the $+N$ control compared to the 1st (Figure 10A and B). When N was supplied on day 23, the 1st trifoliate did not expand beyond that of the $-N$ treatment (Figure 10A), while that of the 2nd trifoliate increased by about 50%. The same response pattern occurred with the other two soybean genotypes.
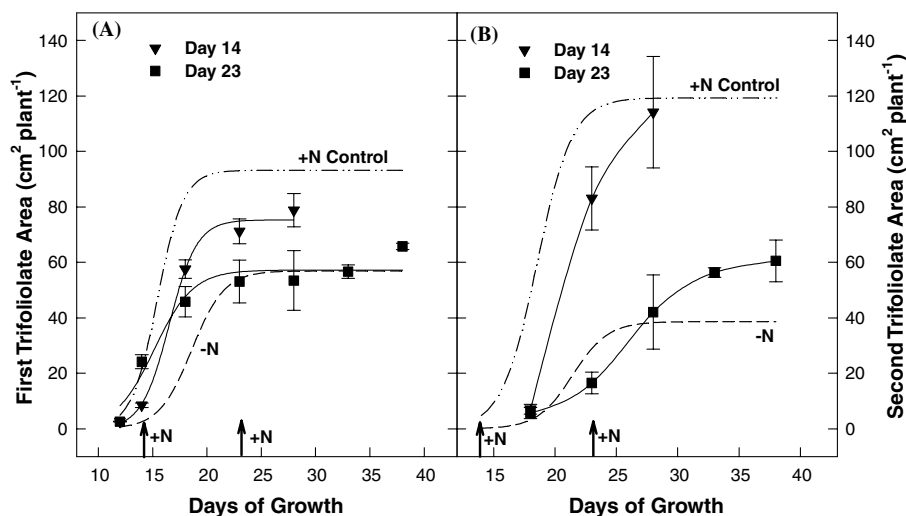
Figure 10. Differences in the expansion of the first (A) and second (B) trifoliolate leaves of soybean line NC-106 when N was added to the nutrient solution on day 14 or 23. Dashed lines represent leaf areas when N was continuously present in or withheld from nutrient solutions (from Figure 5). Vertical bars represent standard error of the mean.

Discussion

Seedling establishment and competitiveness in infertile environments are linked with the ability to adjust to nutrient stresses and take advantage of nutrients that become available in patches or pulses (Caldwell, 1994; Chapin, 1988; Fitter, 1994). The results of these experiments indicated quite clearly that the ability of seedlings to sustain growth in the first weeks after germination was closely tied to the seed N pool. And, the seed N content and the related N status of the tissue affected seedling recovery from N stress when external N was supplied.

The N stress progression

Even though soybean has a large seed with relatively high %N compared to some other species (Wright et al., 1999), growth inhibitions were being expressed in all genotypes within 2 weeks of germination without an external N source. The pattern of N release to vegetative tissues was similar among the genotypes, apparently reflecting the highly regulated breakdown of seed proteins, which occurs in an orderly sequence (Muntz, 1996; Wilson et al., 1986). Because the endogenous N release period was similar, seed with higher N contents transferred larger

amounts of N into the developing tissues over approximately the same time period.

In general terms, the relationship between seed N release and whole plant response can be viewed in a similar conceptual framework as that developed from relative addition rate experiments, where growth of N deficient plants is controlled by the rate of N uptake (Larsson et al., 1992). In our case, N is simply being supplied from an endogenous source. A greater supply from the endogenous source led to more rapid growth and less N stress.

In most studies with older plants that are N deficient, inhibited leaf expansion is the first and most pronounced growth response (Rufty, 1998). The seedlings followed a similar pattern, as restricted expansion of primary leaves was the first indication of N deficiency. Microscopic examinations of the apical meristem indicated that leaf initiation also was inhibited within the same time frame. Higher seed N content ameliorated both effects.

The mechanisms responsible for decreased leaf initiation and expansion are not entirely clear. Anatomical studies of individual leaves with N stressed castor bean (Roggatz et al., 1999) and sunflower (Trapani et al., 1999) have shown that inhibitions of both cell division and expansion are involved. A positive correlation between cell divi-

sion and N supply suggests that lower cell division rates might result directly from lower N availability for DNA replication and protein synthesis. Restricted cell expansion during N stress has been related to decreased hydraulic conductance in roots, which causes lower cell turgor in leaves (Radin and Boyer, 1982). It is unclear, however, if that type of mechanism would occur in these soybean seedlings, where N originates from seed.

Even though higher seed N minimized adverse effects on leaf development during the N stress progression, there was not any obvious restraint on the S/R ratio adjustments that are so crucial to plant survival (Chapin, 1991). It has been seen in many studies with older plants that S/R ratios are lowered soon after a N stress is imposed and the degree of adjustment reflects the degree of stress (Brouwer, 1962; Ingestad, 1979; Rufty et al., 1984). The S/R ratios of NC-105 were at least as low as the other two genotypes once the adjustments occurred, even with the higher seed N status and less severe N stress. The most plausible explanation for S/R growth adjustments in older plants under N stress is that leaf growth is affected more than photosynthesis, which results in spillover of carbohydrate to the root and stimulation of root growth (Rufty, 1998). With the soybean seedlings, leaf growth followed the expected response pattern, being restricted more with lower seed N, so the lack of proportional S/R adjustment appeared due to inhibited root growth in the more N stressed genotypes. It seems reasonable to think that the extra increments of carbon needed to maximize the root growth responses were not available because of the severely limited photosynthetic capacity.

Recovery from N stress

Seed N had a strong influence on the ability of seedlings to recover from N stress. Several aspects of the recovery response should be considered. The first, of course, is the ability of roots to take up N. Upon first exposure to external nitrate, the nitrate transport system must be induced. In past experiments, most done with seedlings of crop species, maximal uptake rates were reached within about 4–12 h (Crawford and Glass, 1998; Glass, 2003; Jackson et al., 1973; Tischner, 2000). In the present experiments, the N-stressed seedlings were exposed to a relatively high level of ^{15}N-nitrate (600 μM) at 15 days, but uptake rates g^{-1} of root did not reach a maximum (full induction) until 4–5 days later (Figure 8A). When unlabeled nitrate was supplied at 23 days, the uptake rate had not reached a maximum even after 12 days (Figure 8B). The relatively slow induction probably was caused by the low N status of the root and impaired protein synthesis; specifically, the formation of functional membrane proteins (Doddema and Otten, 1979; Teyker et al., 1988). Following the induction phase, ^{15}N uptake rates remained high and well above the controls. So, there was no indication of major engagement of the feedback control system (Glass, 2003; Imsande and Tourraine, 1994).

A second key component of recovery from N stress is stimulation of morphological development in the leaf canopy. The results suggested that recovery of leaf expansion was dependent on the stage of leaf development at the time external N was supplied. Leaf expansion at a particular stem position increased when nitrate was supplied early enough during its expansion phase, but expansion was not affected when nitrate was supplied after an expansion plateau was reached under severe N stress. This relationship is similar to that described with N-stressed castor bean (Roggatz et al., 1999) and sunflower (Trapani et al., 1999), where it was proposed that a restriction of cell division prevents additional leaf expansion.

In contrast to the situation with individual leaves, meristematic activity at the shoot apical meristem clearly retained the capability of responding to the external N supply. Although N stress led to a marked down regulation of leaf initiation (Figure 6, Table 2), initiation recovered immediately when external N was supplied and, at least in the two genotypes with higher seed N, initiation rates were similar to +N control plants. That was true whether N was introduced into the system on day 14 or on day 23 when the plants were in advanced stages of stress (Figure 9, Table 2). The quick recovery of leaf initiation suggests that the apical meristem assumed a quiescent state during the N stress progression, and was capable of rapidly increasing cell division rates when N became available. Although no micrographs are shown, the frequent examinations of the apical dome revealed that little structural change was occurring at the growth center, as was reported in the earlier N stress

experiments with sunflower (Trapani et al., 1999) and experiments examining the apical dome of P-stressed soybean (Chiera et al., 2002.)

The examination of leaf initiation at a microscopic level allowed relatively precise definition of the rapid response to external N. Rapid leaf initiation occurred even though nitrate uptake and transport of N to the shoot was slow during the first days of the induction phase. The response could involve at least two factors. One is the delivery of N to the meristem, which could serve as a signal (Crawford, 1995), as well as increasing the availability of N for protein and DNA synthesis. The other is hormonal regulation. When N stress is relieved, decreases in absisic acid and increases in cytokinins have been found (cf. Clarkson and Touraine, 1994).

Larger-scale implications

Treatment solutions without a N source were used to accentuate physiological responses associated with varying seed N contents. It is rare, of course, that field situations would be encountered where N would be entirely absent. Nonetheless, physiological studies have consistently shown that plant responses to N stress are similar and primarily differing in magnitude with the degree of stress (Ingestad and Lund, 1979; Rufty et al., 1984). Thus, it would be expected that differences in seed N content will result in seedling growth responses like those observed here whenever N is available at sub-optimal amounts.

In an agronomic context, the results clearly have implications for soybean. It is common for soybean not to be fertilized with N, even in developed countries, because N_2-fixation can potentially supply adequate N for maximizing yields. Nonetheless, young plants typically experience a period of N stress between the time seed N is depleted and the N_2-fixation system fully develops 4–5 weeks after germination. From the current experiments, it seems that genotypes with higher seed N will have increased vigor and minimize negative impacts during the N-deficiency period. Increased vigor would enhance disease and pest tolerance, competitiveness with weeds, and the ability to avoid stresses such as drought. Also, more vigorously growing seedlings that have greater root mass generally develop more nodules per root length (Smith and Ellis, 1980),

which could shorten the time until optimal N nutrition is obtained.

The differences in seed N may also have implications for non-cultivated plants germinating in low fertility, natural settings. Seed nutrient contents can be extremely different among species groups (e.g. Wright at al., 1999), which may be an important factor explaining differences in early growth and establishment of communities after environmental disturbances. Seed nutrition effects also could be expressed intraspecifically. The fertility of the maternal growth environment can lead to differences in seed nutrient content (Fenner, 1992; Wulff, 1995). It is logical to think that the expression of maternal effects in offspring and offspring competitiveness (Stratton, 1989; Tungate et al., 2002; Wulff et al., 1994) could be associated with responses like those observed in these experiments with soybean.

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